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Cloning and purification of VapB22, VapC22, and VapBC22

KT Krishan Gopal Thakur RS Ramandeep Singh

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An abbreviated version of this protocol was published in Science Advances in Jun 2020

VapBC22 toxin-antitoxin system from Mycobacterium tuberculosis is required for pathogenesis and modulation of host immune response

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Detailed protocol

1. pBAD-VapC22 construct was transformed into *E. coli* Rosetta DE3 and transformants were selected on LB Agar plate containing Ampicillin (100 µg/mL) and Chloramphenicol (25 µg/mL).

2. Next Day, the colonies were inoculated into 10 mL (X3-4 vials) of LB media in 50 mL tubes and allowed to grow overnight at 37 °C with constant shaking at 180 rpm.

3. Following day, inoculate 750 mL LB media in 2 L flasks with appropriate antibiotics (3-4 Flasks-as the yield is very low because of toxicity).

4. The culture was induced at ~0.5 OD600 using 0.2 % L-arabinose and incubated at 37 °C for 2 h (longer incubation led to cell lysis).

5. Cells were harvested using centrifugation followed by lysis using sonication in the lysis buffer (20 mM HEPES pH 8.0, 250 mM NaCl).

6. The lysate was further centrifuged at 14000 g for 45 min at 4 °C and the proteins were purified using HIS-Select Nickel Affinity Gel in gravity column following the manufacturer's protocol (Sigma Aldrich).

7. After binding through affinity resin, 50 mL of lysis buffer was used for wash step.

8. The protein was eluted using Imidazole (step gradients) in the lysis buffer.

9. The quality of purified protein was confirmed using SDS-PAGE analysis by staining with Coomassie brilliant blue.

10. The fractions containing pure proteins were concentrated and dialyzed in a lysis buffer.

11. The purified protein was further purified using Superdex™ 200 Increase 10/300 GL (GE Healthcare) (Cleaned thoroughly using 500 mL autoclaved water followed by equilibration in 20 mM HEPES pH 8.0, 250 mM NaCl).

12. The peak fractions containing proteins were concentrated (Please note that the protein has a tendency to aggregate upon concentration so keep protein concentration < 50 uM).

13. The purified protein was used in *in vitro* RNase assays with cleavage buffer final concentration 10 mM HEPES pH 8.0, 15 mM KCl, 1 mM DTT, and 1µM $MnCl_2$.

PS: The vectors are also available on request

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Thakur, K. and Singh, R. (2020). Cloning and purification of VapB22, VapC22, and VapBC22. Bio-protocol Preprint. bio-protocol.org/prep563.
2. Agarwal, S., Sharma, A., Bouzeyen, R., Deep, A., Sharma, H., Mangalaparthi, K. K., Datta, K. K., Kidwai, S., Gowda, H., Varadarajan, R., Sharma, R. D., Thakur, K. G. and Singh, R.(2020). VapBC22 toxin-antitoxin system from Mycobacterium tuberculosis is required for pathogenesis and modulation of host immune response . Science Advances 6(23). DOI: 10.1126/sciadv.aba6944